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# SCIENCE

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## SOME BIOCHEMICAL PROBLEMS IN BACTERIOLOGY<sup>1</sup>

THE Society of American Bacteriologists stands, primarily, for pure as distinguished from applied bacteriology. In these days when the applications of the science are becoming so immensely important, and therefore so enticing to the investigator, there is danger that our thoughts turn not often enough to the broader aspect of the science, upon which, as a foundation, all of its applications must ultimately rest. We as a society must make it our special duty to see that these foundations are laid broad and firm, upon the very bed rock of truth itself.

We have as a society been interested for some time in the preparation of standard and uniform methods of describing bacterial species. This is of fundamental importance, leading as it does to uniformity of method and completeness and comparability of results. When we couple with this the use of the standard methods of the laboratory section of the American Public Health Association we have gone a long way toward the standardization of our work, and have begun the foundation upon which can be built the science of pure bacteriology.

But we must ever beware that we become not slaves to standardization and uniformity. It is well enough to proceed by standard methods, but we must not be tied by them. We must ever be ready to abandon the old and adopt the new, when the new marks the way of progress.

<sup>1</sup> President's address before the Society of American Bacteriologists at the Washington meeting, December 28, 1911.

At this time I wish to bring to your attention some of the lines along which we are making little progress, I believe, because of the false security which we are taking in our standard methods.

First, suppose we consider our ordinary culture media. We have drawn up ridiculously exact procedures for mixing and boiling and filtering and titrating our broth, agar and gelatin, and when we are through we believe we have a standard, uniform product. But indeed it is not so. Not only are the results obtained in different laboratories unlike, but two lots of the same medium made at different times in the same laboratory are extremely unlike, when measured by the delicate physiological properties of organisms which respond to the slightest of chemical differences. Far better, it is true, are the results secured at the present time by the use of standard methods, than before their introduction, and I do not for a moment want to decry our standard methods, but I simply want to warn against the false security which their use may give. For however careful we may be in the process, the final result can never be uniform as long as the ingredients used are themselves variable. No medium can be standardized, that is, can be exactly duplicated at another time or place, if it contains such variable materials as beef extract, either freshly made or commercial, peptone, gelatin, agar, blood serum, bile, etc. I am inclined to think that in order to get uniform results, particularly in our study of the delicate physiological properties upon which we depend so largely for the differentiation of bacterial species, the time has come for us to abandon altogether the use of all complex and variable animal and vegetable products, and in their places to substitute materials of definite, known, chemical composition. From my work of

the past few years I am led to believe that for every organism it is possible to prepare a synthetic medium containing chemically pure salts, upon which these organisms will grow and grow well. Such a medium as this we are able to duplicate exactly anywhere and at any time.

In the past we have been inclined to think that the physiological properties of many organisms were too variable to be of much use in species determination. I am coming more and more to think that it is not so much the properties of the bacteria which are variable as the environment in which we have attempted to study them. We talk about rejuvenation of organisms to restore them to normal conditions. Our attempts at rejuvenation are attempts to make normal organisms adapt themselves in short order to an abnormal environment. Could we but supply the proper environment we should find the organisms responding in an entirely normal and uniform manner. It is the environment, our culture media, that need rejuvenating and not the organisms. This rejuvenation will come about, I believe, through the adoption of synthetic media of absolutely known chemical composition. Then the physiological properties of organisms will come into their proper place in species differentiation, for then we can substitute the exact qualitative and quantitative tests of the chemist for the inexact determination of the present-day bacteriologist.

There is scarcely a physiological property of the bacteria that is to-day accurately measurable. Variations in the media are so great that measurements at present amount to nothing. Chemistry advanced to its present position as a science only when it became quantitative. Lord Kelvin said: that "When you can measure what you are speaking about, and

express it in numbers, you know something about it; but when you can not measure it, when you can not express it in numbers, your knowledge is of a meager and unsatisfactory kind; it may be the beginning of knowledge, but you have scarcely in your thoughts advanced to the stage of science." Bacteriology must then become quantitative before the real foundations of the science are laid, before it can take its proper place among the sciences. And it can not become quantitative until we can measure its reactions in media of known chemical composition, by methods as exact and definite as any known to the chemist. We are always dealing, it is true, with living protoplasm, but if we place it under definitely determined environment, recent experiments lead us to believe that living substance always responds in a perfectly definite way both qualitatively and quantitatively.

The very complexity of the society's card for the description of bacterial species is to me its own condemnation. It is our admission of ignorance. Are not the real diagnostic characters of a species lost in a maze of unessential characters? Aside from the morphological data, which I believe is all-essential, the cultural and biochemical data could, I am sure, be simplified to a very considerable extent as far as species differentiation is concerned. The forms of cultures and colonies are but functions of the morphology and methods of subdivision of the organisms themselves, as has been so well shown by the recent work of Graham-Smith.<sup>2</sup> We might then eliminate from the card many of the complex descriptions of cultures and colonies on agar and gelatin, retaining perhaps the agar streak and the gelatin stab, and sub-

stitute therefore information in regard to their determining factors. And then among the biochemical data if we could eliminate all but a few deep-seated physiological characters which are accurately measurable, and which can be easily determined by means of accurate chemical tests on synthetic media of known composition, we would have a simple, accurate, all-sufficient description of a bacterial species.

One of the weakest parts of bacteriology to-day is its taxonomy. Our methods of classification of bacteria are practically the same to-day that they were in the earliest days of the science. Migula, it is true, systematized the scheme of classification to a certain extent, Chester contributed to its more accurate terminology, and the Winslows gave it a new impetus by the introduction of the methods of biometry. But when we think of the thousands of described species among which but two or three genera are recognized, it must be apparent at once that some of our generic names are seriously overworked. In other biological sciences the classification into species, genera, families, orders, classes, etc., is not only of great convenience, but it also expresses for us something of the relationship of the different groups, something of their probable ancestry and line of evolution. I see no reason why the bacteria should not be classified in the same way. The bacteria are not exceptions to the general biological laws. Variation, selection, heredity, are the factors of evolution here as elsewhere. It is true among the unicellular forms we are free from many of the complications which enter into our discussions of the origin of species among multicellular animals and plants. Sexual reproduction is absent, there is no differentiation into germplasm and somatoplasm to prevent the acquisi-

<sup>2</sup> Graham-Smith, G. S., "The Division and Post-fission Movements of Bacilli when Grown on Solid Media," *Parasitology*, 3, 1910, 17.

tion of new characters, the environment presses very closely upon these unicellular forms and they respond more directly to it. Generation follows generation with startling rapidity, elimination of the unfit proceeds rapidly, the struggle for existence is more severe because of the enormous numbers concerned. In the bacteria we ought almost to be able to see the actual process of evolution since we can place under observation untold numbers of generations as compared with the comparatively few generations of multicellular forms which we are able to observe. Eons of multicellular time are literally compressed into a few unicellular days.

Because of this lack of the regulating influence of reproduction and the greater influence of the environment and the rapidity of reproduction, we might expect to find among unicellular organisms a series of intergrading forms without divisions into groups that resemble the species of higher forms. But this is not so. Among the protozoa differentiation has followed definite lines, and classes, orders, families, genera and species are well marked. The species of protozoa are, for the most part, based on morphological differences, it is true, but among the bacteria a morphological basis of classification fails us. The only morphological differences are the three main divisions as to shape, the round, the rod and the spiral, with slight modifications as to size, arrangement of flagella, formation of spores, chemical composition, etc. But in spite of this lack of morphological basis for classification we find as distinct groups as among the protozoa. As the Winslows say:<sup>3</sup>

Typhoid germs descend from typhoid germs, tubercle bacilli from tubercle bacilli. The same

yellow coccus falls on gelatin plates exposed to the air all over world. The same spore-forming aerobes occur in every soil, the same colon bacilli crowd the intestines of animals and man in every clime. These fundamental types can not be transformed into each other.

And yet to a considerable extent these fundamental types are based not on morphology, but upon physiological differences.

It is among the bacteria that for the first time among living forms we find physiological differences made a basis for classification. Are physiological properties valid criteria for the separation of species? We are accustomed to think of morphological characters as fairly stable, rather difficult to modify, but of physiological characters as easily modified and directly dependent upon the environment. But have we any reason to assume that physiological characters are not deep seated also, are not stable, fully as much as morphological characters? Are we not in reality dealing with characters of the same sort as morphological characters except that we can not see their ultimate basis in structure? I am not sure but what physiological characters are even of greater importance than those of form and external appearance, especially in such simple and undifferentiated forms as the bacteria, since they testify to deep modifications in the chemistry and vital properties of the protoplasm itself. It is upon such chemical properties as these that these simple organisms depend for their very existence, and not upon a modification of external appearance.

But before we can proceed far in the use of physiological differences for species determination we must be able accurately to determine these delicate characters. Hitherto this has been impossible because of the variable and uncertain culture

<sup>3</sup> Winslow and Winslow, "Systematic Relationships of the Coccaceæ," p. 1, John Wiley and Sons, 1908.

media in which we have attempted to study them. With the adoption of media of definite chemical composition for making our determinations and measurements, the physiological characters of the bacteria will assume the importance of the morphological characters of higher organisms. Then and then only shall we be able to arrive at a natural classification of bacterial species which shall express for us their true relationship.

With the adoption of such accurate chemical tests of the physiological characters of the bacteria much of the present apparent variation will pass away and we shall find the physiological characters of the different groups as stable as any of their morphological characters. And what little variation remains—and we shall always find some variation as long as we deal with living organisms—we can handle easily by the method of biometry. For, as you know, the methods of statistical variation can apply only in characters which are measurable.

This matter of better methods of species identification and a new taxonomy will be one of the first outcomes of the adoption of simple chemical culture media.

Another important result will be the increased ease with which certain biochemical problems in bacteriology can be attacked. Think for a moment of our endeavors to find a suitable method of isolating the colon bacillus. Litmus-lactose-agar, endo, esculin, neutral red, malachite green, phenol, bile and bile salts and their various combinations are but expressions of our total ignorance of the chemistry involved. The problem should be attacked in an entirely different way. First, we should determine the simplest synthetic medium upon which the colon bacillus will grow rapidly and well. Then by adding to it the chemical body which is the in-

hibiting agent in the phenol or in the bile we ought to have the ideal medium for colon isolation. A beginning along this line has been made by Dolt<sup>4</sup> in his ammonium lactate, glycerin and malic acid media. But further work must be done until we have a simple synthetic substitute for the complex, variable and inconvenient media upon which we depend so much at present.

And then take the differentiation of the colon and typhoid groups by cultural characters. There must be certain chemical differences inherent in these organisms which could be easily determined by the use of synthetic media adapted to each organism.

Another desideratum is a simple synthetic medium to be used as a substitute for blood serum when used for the diagnosis of diphtheria. We have to depend at present upon the uncertain and unsatisfactory supply of blood from the abattoirs. How much better would it be to find a chemical substitute which would be just as certain for diagnosis and much easier to prepare? I believe that such a substitute will soon be forthcoming.

We have been gradually accumulating a knowledge of a considerable number of important chemical products which are produced by the activities of bacteria either by synthesis or decomposition. Many of these substances are of great importance. Many are produced only as far as we know at the present time by bacteria. How little at present we know of the chemistry of these products! How much can be learned by a study of the formation of these substances in culture media of known composition! The chem-

<sup>4</sup> Dolt, M. L., "Simple Synthetic Media for the Growth of *B. coli* and for its Isolation from Water," *Journal of Infectious Diseases*, V., 1908, 616.

istry of ptomaine and toxin formation, of pigment formation, of enzyme production, may be worked out in this way. The chemist laughs at our present methods of testing the production of gas, the reduction of nitrates, the production of indol, the fixation of nitrogen. And yet when these processes are tested in synthetic media how simple are the chemical tests involved and how accurate may be the results!

Slowly and laboriously the physiological chemist is now trying to work out the chemistry of protoplasm, of the proteins, such as the albumens, the peptones and proteoses. His principal line of attack is by a study of their decomposition products. The brilliant work of Fischer opened up an entirely new field of research when he undertook the study of the synthetic production of the polypeptides from amino acids by an amide link. Still more light might be thrown upon this important problem by the study of the growth of bacteria in simple chemical solutions. For in the synthetic culture medium we would be able to study step by step the synthesis of protein under conditions accurately controlled and completely known. For when bacteria are growing on simple chemical media and are building up untold millions of bacterial bodies from the simple salts present, we can almost see protoplasm in the making.

And finally, aside from the important chemical information which may in this way be obtained, I believe that some most interesting biological information lies along this path. Who would dare to deny that some day it might be possible by some such method as this to discover the secret of the very origin of life itself!

These then are some of the lines of work which appear to me to mark progress in the science of pure bacteriology. Brilliant

as may be the results of the study of the applications of bacteriology, fully as interesting, and hardly less important, will be the results that come from the application of exact chemical methods to our at present inexact and rather uncertain bacteriological procedures.

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*THE TEACHING OF MICROBIOLOGY IN  
COLLEGES OF UNITED STATES  
AND CANADA*<sup>1</sup>

IN his admirable presidential address delivered before the Society of American Bacteriologists at the Ithaca meeting in December, 1910,<sup>2</sup> Professor V. A. Moore made a strong argument for bacteriology as a science for general culture as well as for professional value, and a vigorous appeal for better teaching and more carefully developed courses. As a result of this exposition of the subject a committee of this society was appointed to make a systematic inquiry into the teaching of microbiology, including bacteriology, protozoology and the study of the lower fungi, in the educational institutions of the United States and Canada.

The purpose of the inquiry is primarily to learn to what extent bacteriological instruction is given in these institutions, and secondarily to note the character of the teaching in these subjects and the scope of the work. It is possible that as a result of these data submitted the committee may be able to formulate a generally broad and satisfactory plan of instruction in this subject, although this is not the immediate purpose of the committee and it is possible that an attempt to do so would react harmfully in certain instances.

While it is hoped the inquiry will eventually embrace institutions of all ranks, the first report of progress presented at the Washington meeting dealt only with the colleges and

<sup>1</sup> An abstract from the first report of progress by the Committee on Education of the Society of American Bacteriologists.

<sup>2</sup> SCIENCE, N. S., Vol. XXXIII., No. 843.